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Long-term changes in abundance and diversity of tintinnids in the Gulf of Trieste (Northern Adriatic Sea)

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Abstract

Abundance and composition of the planktonic tintinnid ciliates were studied in the Gulf of Trieste (Northern Adriatic Sea, NE Mediterranean Sea) from July 1998 to July 2016. Tintinnids were collected biweekly-monthly from the LTER station C1 (200 m offshore, 17.5 m depth) at four depths (surface, 5 m, 10 m and 15 m). The maximum tintinnid abundance reached 4476 ind. L^{-1} at surface in February 2016. The tintinnid community comprised a maximum of 35 species and was dominated by the genera *Stenosemella*, *Tintinnopsis*, *Codonellopsis*, *Salpingella* and *Eutintinnus*. The most abundant species were *Stenosemella nivalis*, *Tintinnopsis nana*, *Codonellopsis schabi*, *Salpingella rotundata* and *Eutintinnus apertus*. We found a species-specific correlation with the abiotic factors considered, i.e., temperature and salinity. Temperature was positively correlated with *S. rotundata* and *E. apertus* and negatively with *S. nivalis*. Salinity was negatively correlated with the majority of the detected species. Agglutinated species presented winter maxima while hyaline species showed higher abundance in summer-autumn. Some key species were present over the whole period studied. Significant differences within the water column were not seen in the species composition, but were seen in the relative abundances of the same species at different depths. *Stenosemella nivalis*, *S. ventricosa* and *Tintinnopsis beroidea* can be considered as keystone species in the area and their possible loss can be seen as a signal of changes in the structure of the entire planktonic system.

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Keywords
Tintinnids, Adriatic Sea, LTER

Introduction

Tintinnid ciliates represent a fraction of microzooplankton (broadly heterotrophic planktonic organisms spanning 20 μm to 200 μm). They have a key position in the plankton food web as major consumers of picoplankton and nanoplankton and serve as prey for metazoans (Capriulo et al. 1991). Although they represent only a small part of the planktonic ciliate community (generally less than 10% of biomass), they can be very important in a variety of ecosystems (Dolan 2000). In coastal waters, they are occasionally abundant (Kršinić 1987, Modigh and Castaldo 2002) and are reported to ingest up to 27% of the annual primary production (Capriulo and Carpenter 1983, Verity 1987). Tintinnids are ideal for studying changes in the structure or composition of the plankton communities (Thompson et al. 1999). In fact, they are recognised from the lorica morphology, a quite simple feature that ensures homogeneity in the taxonomic identification with a low risk of misclassification when different researchers are involved over the years in long-term studies.

Tintinnids are the most investigated component of microzooplankton communities in the Mediterranean Sea where the west-to-east increase in species richness and in taxonomic diversity has been well documented (Dolan 2000, Dolan et al. 2002, Modigh and Castaldo 2002, Gómez and Gorsky 2003, Sitran et al. 2007, 2009). Previous studies in the Adriatic Sea have shown that tintinnids are an important plankton fraction, characterised by variable abundances along the basin (Lipej 1992, Fonda Umani et al. 2010, Moscatello et al. 2004, Bojanić et al. 2006, 2012, Kršinić and Grbec 2006). For this area, studies on tintinnids have focused both on their seasonal distribution (Fonda Umani et al. 2005a, Milani et al. 1991, Monti et al. 2012) and trophic role (Fonda Umani and Beran 2003, Fonda Umani et al. 2005b).

The Gulf of Trieste is the most northern part of the Adriatic Sea, with a surface area of about 600 km^2 and a maximum depth around 23 m (Malej and Malačič 1995). The main freshwater input in the Gulf is through the Isonzo River and the hydrodynamics is driven by a wind regime characterised by strong wind events, by the interaction with the Adriatic circulation and by seasonal shifts from stratification to mixing (Cossarini and Solidoro 2007). The circulation pattern is variable; however, the prevailing East-North-East wind is the most efficient driver for the water exchange (Solidoro et al. 2009). Over the last decade, an increase in seawater temperature and salinity was detected for the area, particularly at the surface, and a reduction in nitrate and silicate was recorded (Malačič et al. 2006, Mozetič et al. 2012). The area remains phosphorus-limited with P concentrations always low, ranging from 0.05 to > 3 μM (Fonda Umani et al. 2007, Lipizer et al. 2011). The satellite-based chlorophyll concentrations indicated an oligotrophication trend from 1998 to 2007 (Mozetič et al. 2010). More recently, Giani et al. (2012) reported an increase in phytoplankton biomass since 2007.

Biological factors, such as primarily prey abundance and predator presence, influence tintinnid behavior and ecophysiology (Montagnes 2013). Nevertheless, species diversity is not always dependent on food availability and quality or predator abundance (Dolan and Gallegos 2001), and abiotic factors can also be important in shaping tintinnid communities. The influence of salinity on tintinnids has been generally overlooked because of their euryhaline distribution and their ability to survive over a large range of salinity values (Montagnes 2013). Temperature may influence the tintinnid ingestion rate, growth and cell size (Montagnes 2013). Very few data exist on tintinnid diversity and their relationship with abiotic factors such as salinity and temperature, especially for the Adriatic Sea.

In this paper, we analysed the long-term development of tintinnids collected in the Gulf of Trieste over a period of 18 years. The tintinnid assemblages were studied in terms of abundance and diversity along the water column and in relation of temperature and salinity. The main goal of this study was to identify the main patterns of temporal variability of the whole tintinnid community and its main components, and the possible key species.

Materials and Methods

Sampling method

Sampling was carried out in the Gulf of Trieste aboard different boats from July 1998 to July 2016 at the LTER station C1 (https://deims.org/96969205-cfdf-41d8-979f-ff881ea8dc8b). The sampling station is located 200 m offshore at 45°42'3"N, 13° 2'36"E (bottom depth 17.5 m) (Figure 1). Water samples were collected early in the morning, biweekly (from June 2002 to August 2005) or monthly (the rest of the period) at four depths (0 m, 5 m, 10 m, and 15 m), using Niskin bottles. Two litre samples were transferred into polycarbonate bottles and immediately preserved with formaldehyde buffered with $CaCO_3$ (4% final concentration). Temperature and salinity data were recorded by a CTD probe (Idronaut Ocean Seven 316 and SeaBird 19 Plus) not connected with the Niskin bottles.

Sample processing and analysis

In the laboratory, the samples were allowed to settle for 3 days, after which the initial 2 L volume was reduced to 200 mL by siphoning through a glass tube made specifically for this purpose, under a fume hood. The reduced samples were stored in dark glass bottles and subsamples (50 mL) were analysed using inverted microscopes (Leitz and Leica DMI 3000B) at x 200 magnification following the Utermhöhl method (Utermöhl 1958). The entire sedimentation chamber was analysed and tintinnid abundance was expressed as number of individuals per liter (ind. L^{-1}). We counted a variable num-

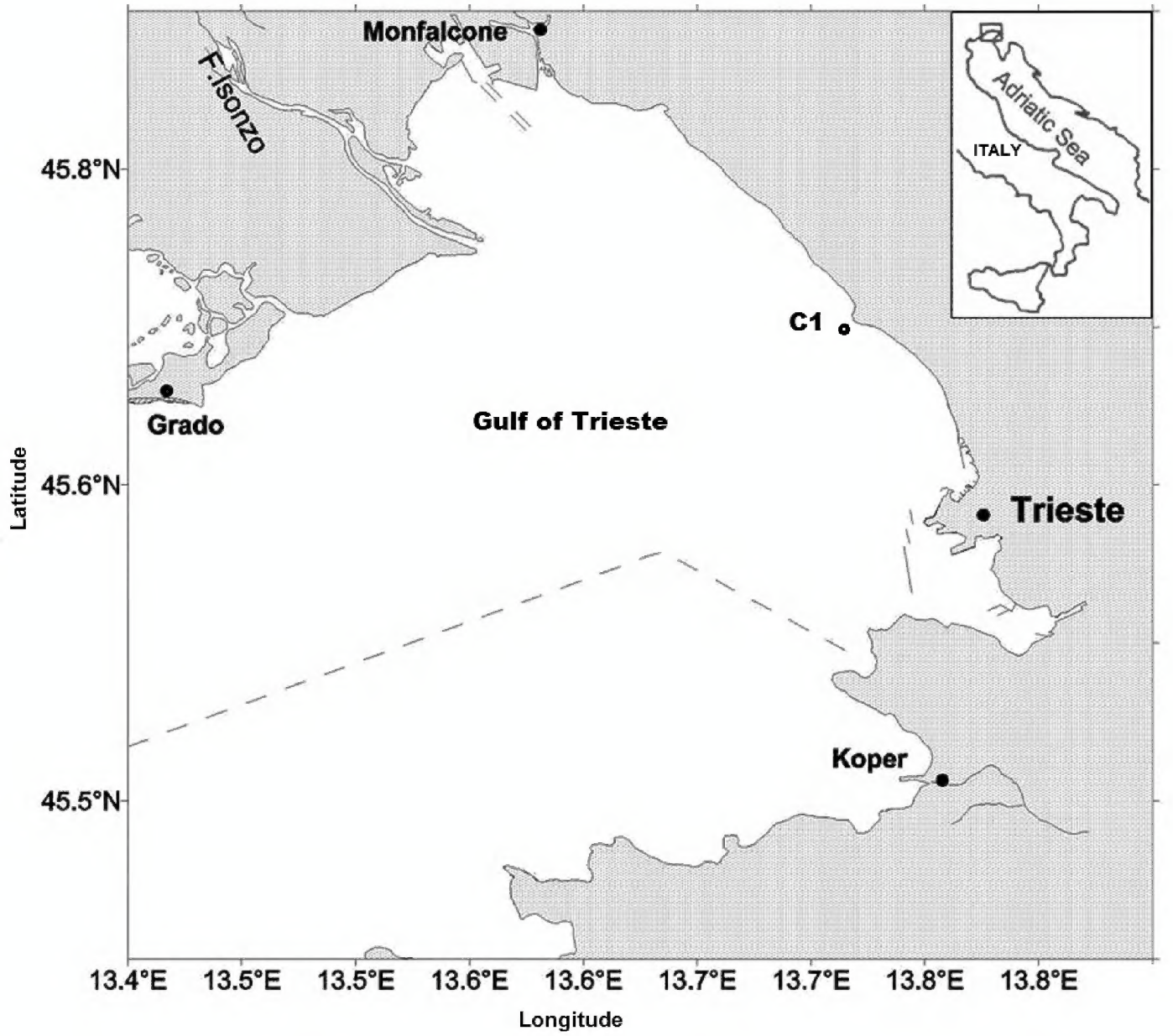


Figure 1. Map of the Gulf of Trieste showing the location of the LTER sampling station C1.

ber of tintinnids in each sample, from the minimum of 0 to the maximum of 2238 tintinnids (median value 16 tintinnids), without any replication. The samples were analysed within one month of the collection date. A total of 1000 samples were analysed over 18 years. For estimating the abundance within the water column (0–15 m), the integrated values were calculated following the trapezoidal rule (Walsby 1997).

Tintinnids were determined according to the classifications by Kofoid and Campbell (1929), Rampi and Zattera (1982) and Kršinić (2010a). Taxonomic criteria were maintained homogeneous over the whole period by a series of inter-calibration exercises among the different researchers (six), under the supervision of the responsible taxonomist (M. Monti-Birkenmeier). The final classification followed Kršinić (2010a) and species names were updated according to the most recent nomenclature. In particular, *Amphorella quadrilineata* was changed in *Amphorides quadrilineata*, *Amphorella quadrilineata* var. *minor* in *Amphorides quadrilineata* var. *minor*, *Dictyocysta elegans* var. *speciosa* in *Dictyocysta elegans*, *Favella campanula* in *Favella adriatica*, *Tintinnopsis levigata* in *Tintinnopsis cylindrica*, *Tintinnopsis minuta* in *Tintinnopsis nana*. Furthermore,

species detected less than twice over the period were grouped to the genus or order level (*Amphorellopsis tetragona* to *Amphorellopsis* sp.; *Codonella aspera* to *Codonella* sp.; *Tintinnopsis baltica* and *Tintinnopsis bütschlii* to *Tintinnopsis* spp.; *Craterella* sp. to Choreotrichida unid.). Empty loricae of tintinnids were not differentiated from filled ones because tintinnid protoplast is attached to the lorica by a fragile strand that detaches easily during collection and fixation of the samples. Furthermore, it is not possible to detect the presence/absence of the cell inside for the agglutinated loricae.

Statistical analysis

Statistical analysis was carried out using the PRIMER-7 software package (Anderson et al. 2008) to find out about similarity among years and seasons and to estimate tintinnid species richness and diversity in each sample. ANOSIM and pair-wise tests were performed to analyse the similarity pattern in the dataset considering the factors depth, month and year. In order to test the influence of season, salinity and temperature on the community, we performed Permutational Multivariate Analysis of Variance (hereafter called PERMANOVA) (Anderson and Walsh 2013) on square-rooted Bray-Curtis dissimilarity matrix for the abundance of each taxon. On the same matrix, a distance based Redundancy Analysis (dbRDA) was performed considering temperature and salinity as environmental variables. The significance of the two RDA axes and of the environmental variables considered in the analysis was tested using "vegan" package (Oksanen et al. 2016) in R environment while for the Indicator Value (IndVal), which measured species-season association, considering the relative abundances and the relative frequency in a seasonal sub set of samples, the "labdsv" package was used (Dufrêne and Legendre 1997). The IndVal test was used to test which species were representative for each season and to test the significance of this relationship over the considered period. The box plots and Spearman's correlation coefficients between species and abiotic factors were calculated using the STATISTICA 8.0 software.

Results

Tintinnid abundance

The total tintinnid abundance ranged from 0 to 4476 ind. L^{-1} (February 2016) and the median values ranged from 22 ind. L^{-1} (surface) to 26 ind. L^{-1} (bottom) from July 1998 to July 2016. Tintinnid abundance integrated along the water column showed a high peak at the beginning of the series (775 ind. L^{-1} in October 1998) and decreased afterwards remaining < 250 ind. L^{-1} from March 2000 to April 2007 (Figure 2). Since May 2007, a slow but continuous recovery of tintinnid abundance was registered up to the highest peak recorded in February 2016 (856 ind. L^{-1}).

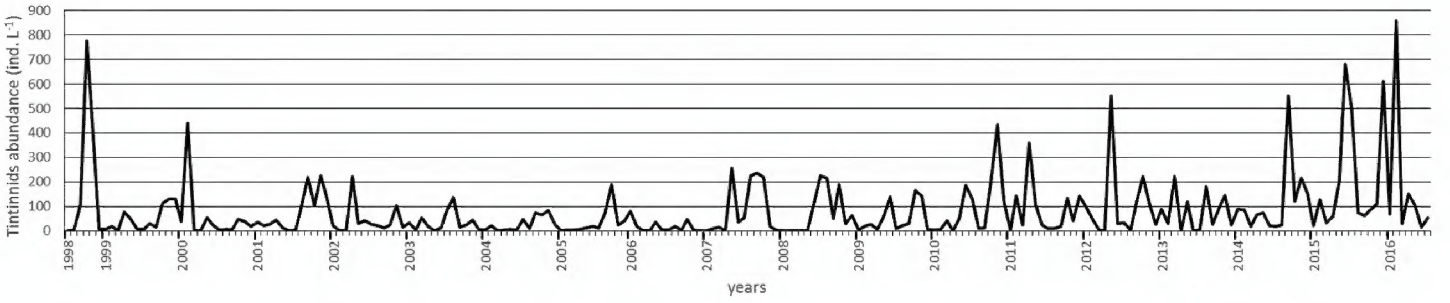


Figure 2. Long-term fluctuations of total tintinnid abundance (ind. L^{-1}) integrated within the water column (0–15 m) from July 1998 to July 2016 in the Gulf of Trieste.

The average seasonal pattern of tintinnid abundance in the integrated water column showed the lowest median values from January to April, and the highest ones from September to November (Figure 3). The pattern was different considering the average abundance for agglutinated and hyaline species. Agglutinated species presented winter maxima whereas hyaline species showed the highest abundance during summer-autumn (Figure 4).

The pair-wise test of PERMANOVA revealed significant differences (p-perm < 0.05) among the different sampled layers, with the exception of 5 m and 10 m depths that showed the highest similarity (Table 1). The highest values of tintinnid abundance were registered at 0 m and 15 m depths (Figure 5). At intermediate depths, the abundances were higher than 800 ind. L^{-1} only in October 2011, May 2012 and July 2015 (Figure 5).

Temporal and vertical patterns of the main genera and species of tintinnids

During the present study, tintinnids belonging to 19 genera and 35 species were recorded. Twenty of these were open sea species and 15 were estuarine-neritic species. The species list shows the frequency of occurrence and maximum abundance of each taxon (Table 2). Tintinnids were dominated by the agglutinated genera *Stenosemella* (*S. ventricosa* and *S. nivalis*) and *Tintinnopsis* (e.g., *T. nana*, *T. beroidea* and *T. radix*) and by *Codonellopsis schabi*. These species present a lorica covered by particles, are more abundant in coastal areas and are considered neritic. The most frequently found hyaline species belonged to *Eutintinnus* and *Salpingella* genera, with open sea species as *E. apertus*, *E. fraknoi*, *S. decurtata* and *S. rotundata* (Table 2). The pair-wise test showed a significant difference (p-perm < 0.05) among most of the years and we did not detect any clear trend in the similarity matrix for the studied period. The highest similarity (17.60) was registered between 2015 and 2016 (Suppl. material 1).

The number of tintinnid species detected in each year from 1998 to 2016 varied between 13 (2006) and 24 (2008) (Table 3). In most of the years, the number of species was very similar with an average species number of 18 ± 3 stdev. Only three species were present in all the years: *S. nivalis*, *S. ventricosa* and *T. beroidea*. A group of six species (*S. rotundata*, *E. fraknoi*, *E. tubulosus*, *T. nana*, *T. campanula* and *S. decurtata*) was

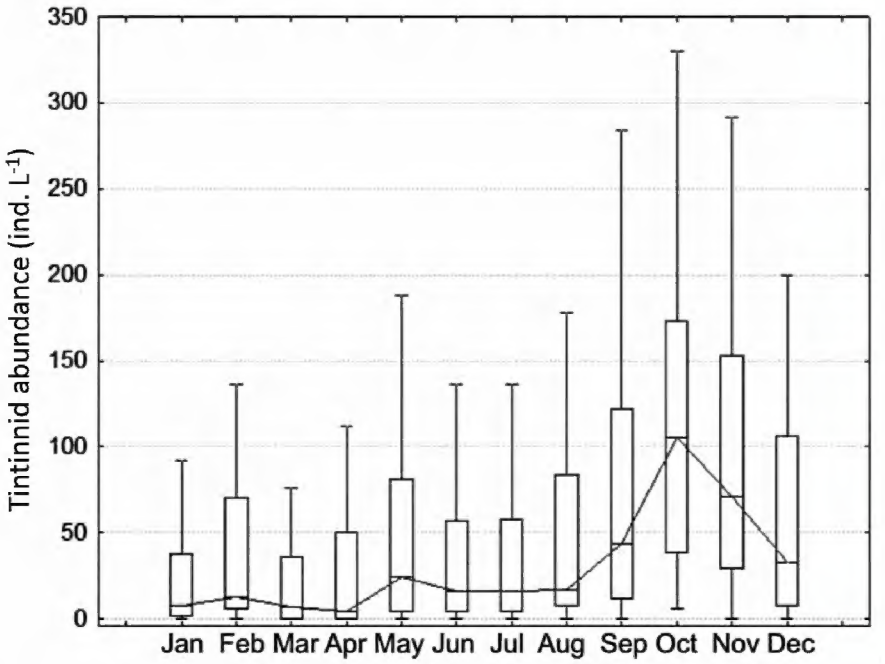


Figure 3. Box plots of monthly averaged abundance of tintinnids (ind. L^{-1}) integrated within the 0–15 m water column. The black lines represent the median, the white rectangles represent the dispersion of the data (25–75%), and the black bars show the non-outlier range.

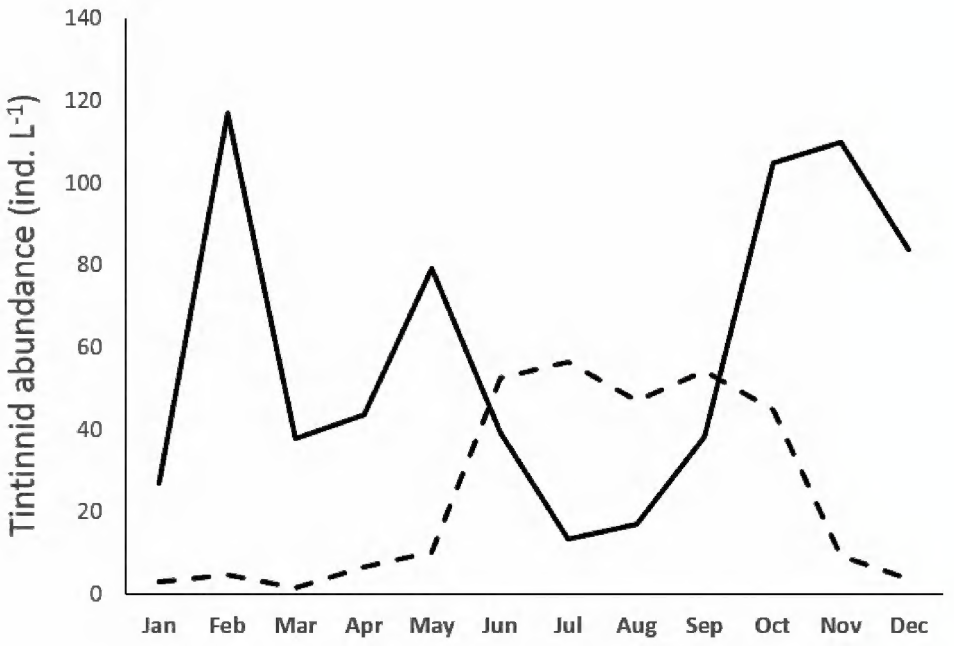


Figure 4. Monthly averaged abundance (ind. L^{-1}) of agglutinated (black) and hyaline (dashed) tintinnids integrated within the 0–15 m water column from July 1998 to July 2016.

Table 1. Results of pair-wise tests of PERMANOVA for the factor depth, built on similarity (Bray Curtis) matrix of tintinnid composition.

Groups	*t*	*p* (perm)
0–5 m	1.61	0.004
0–10 m	1.80	0.002
0–15 m	2.10	0.001
5–10 m	1.15	0.192
5–15 m	2.02	0.002
10–15m	1.45	0.013

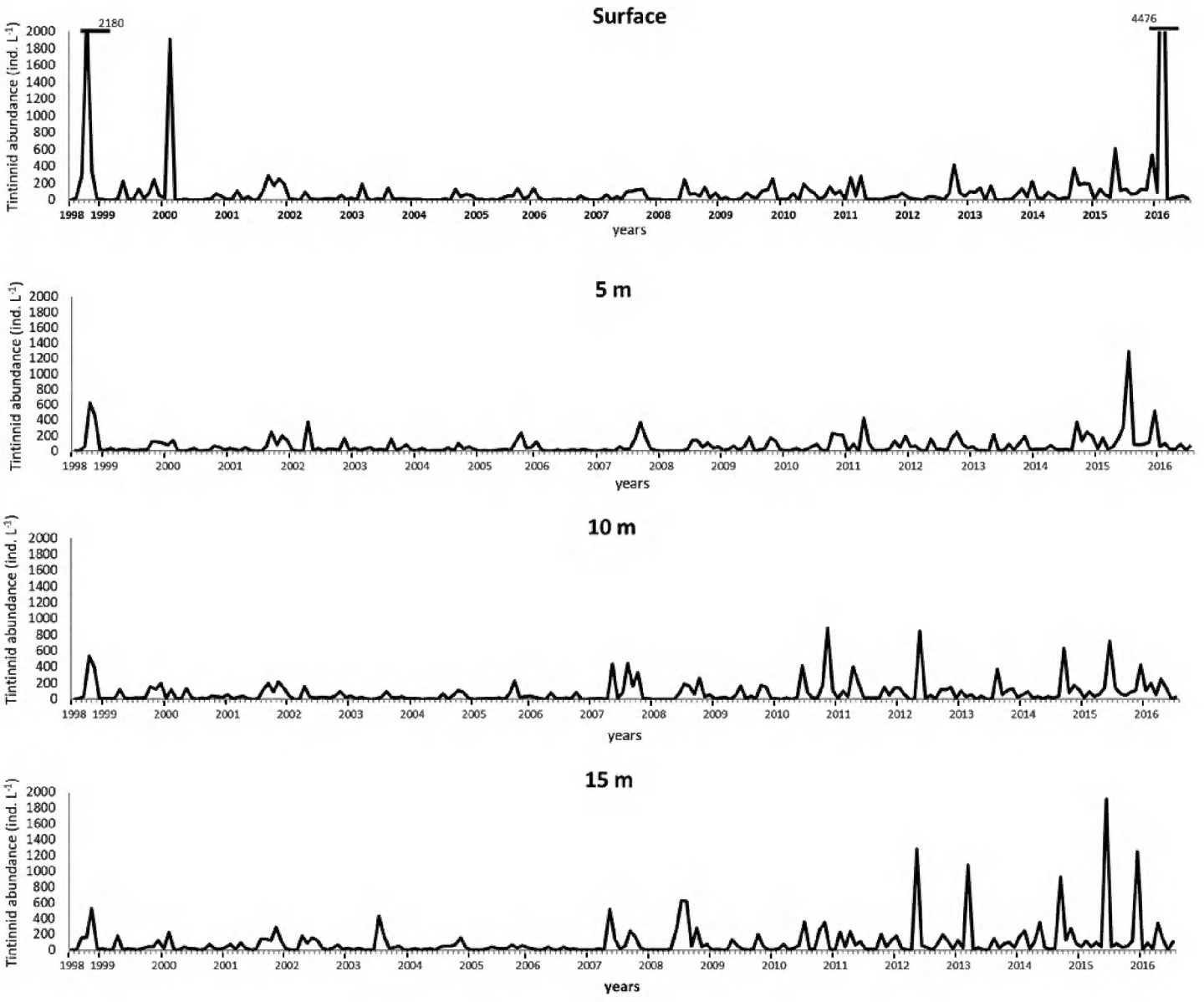


Figure 5. Long-term fluctuations of total tintinnid abundance (ind. L^{-1}) at different depths (surface, 5 m, 10 m and 15 m) from July 1998 to July 2016 in the Gulf of Trieste.

absent only in few years (1–3 y). Five species were detected only in two years or less and should be considered rare taxa (*Dictyocysta lepida*, *Rhabdonella spiralis*, *Protorhabdonella curta*, *Favella adriatica* and *Amphorides* cfr. *amphora*). In particular, *D. lepida* was detected only at the end of the considered period (2015). Conversely, the presence of *Tintinnopsis parvula* and *T. radix* was not registered after 2010.

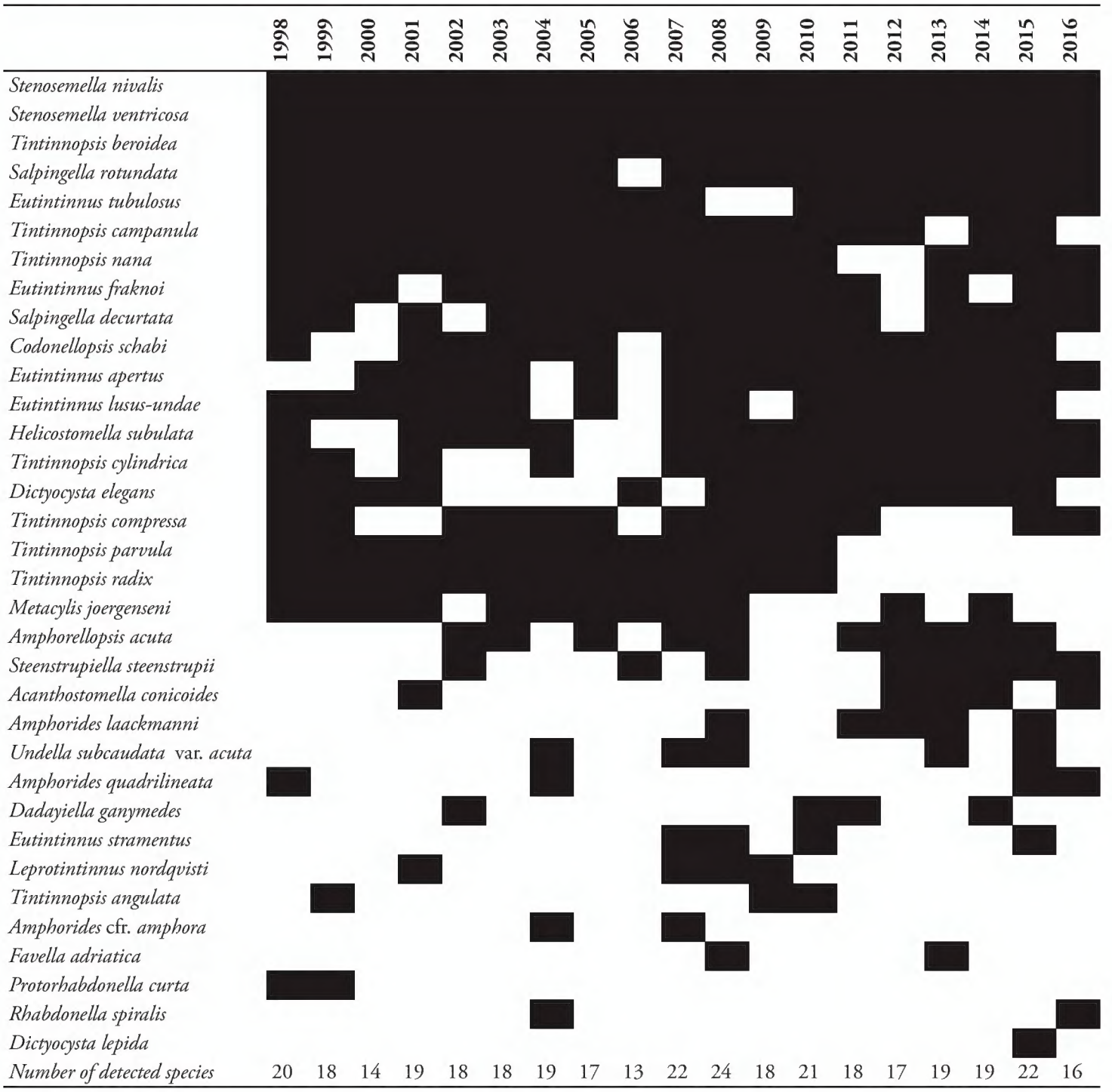
Considering the vertical distribution of the key species, the light differences detected among layers were due to the different abundances of the most representative species (*S. nivalis*, *S. rotundata*, *S. ventricosa*, *T. nana*, *C. schabi* and *Salpingella decurtata*) regardless the stratification of the water column. *Stenosemella nivalis* was absent at surface only in 1998 while *S. ventricosa* and *T. beroidea* were absent in many occasions at different depths (Suppl. material 2). Among the rarer species, *D. lepida* was detected at all depths only in 2015. *Amphorides* cfr. *amphora*, *P. curta* and *R. spiralis* were never collected at the surface. *Rhabdonella spiralis* was never collected at 0 m and 5 m, *P. curta* at 10 m and *A.* cfr. *amphora* at 15 m. *Favella adriatica* was collected only in 2008 and 2013 at different depths (Suppl. material 2).

The number of tintinnid species was lower during winter time, started to increase in spring and reached the maximum in summer-autumn. The specie richness was al-

Table 2. Occurrence (number of samples in which the species was present), frequency, maximum abundance (ind. L^{-1}) and distribution of each tintinnid taxon recorded in the Gulf of Trieste between 1998 and 2016 (total number of samples = 1000). C, common (20–100%); QC, quite common (8–20%); QR, quite rare (3–8%); R, rare (1–3%); RR, very rare (<1%), following the classification of Travers (1971). NES, neritic-estuarine species; OSS, oceanic or open sea species, following Kršinić (2010a, b).

	Occurences	**Frequencies**	**Max abundance**	**Neritic or Open sea**
Acanthostomella conicoides	39	QR	60	OSS
Amphorides cfr. *amphora*	3	RR	4	OSS
Amphorides laackmanni	9	RR	28	OSS
Amphorides quadrilineata	19	R	20	OSS
Amphorides quadrilineata var. *minor*	4	RR	20	OSS
Amphorellopsis acuta	66	QR	66	OSS
Amphorellopsis sp.	2	RR	2	OSS
Codonella sp.	19	R	160	OSS
Codonellopsis schabi	126	QC	444	NES
Codonellopsis sp.	34	QR	35	NES
Dadayiella ganymedes	4	RR	8	OSS
Dadayiella sp.	1	RR	2	OSS
Dictyocysta elegans	50	QR	28	OSS
Dictyocysta lepida	9	RR	12	OSS
Eutintinnus apertus	95	QC	604	OSS
Eutintinnus fraknoi	100	QC	208	OSS
Eutintinnus lusus-undae	68	QR	380	OSS
Eutintinnus strammentus	16	R	28	OSS
Eutintinnus tubulosus	96	QC	650	OSS
Eutintinnus sp.	17	R	208	OSS
Favella adriatica	4	RR	4	NES
Favella sp.	6	RR	24	NES
Helicostomella subulata	67	QR	972	NES
Leprotintinnus nordqvisti	11	R	10	NES
Metacylis joergenseni	39	QR	48	NES
Metacylis sp.	79	QR	512	NES
Protorhabdonella curta	2	RR	10	OSS
Protorhabdonella sp.	7	RR	4	OSS
Rhabdonella spiralis	2	RR	6	OSS
Salpingella decurtata	149	QC	156	OSS
Salpingella rotundata	194	QC	1280	OSS
Salpingella sp.	98	QC	120	OSS
Steenstrupiella steenstrupii	16	R	24	OSS
Steenstrupiella sp.	1	RR	2	OSS
Stenosemella nivalis	429	C	4476	NES
Stenosemella ventricosa	212	C	360	NES
Tintinnopsis angulata	5	RR	38	NES
Tintinnopsis beroidea	122	QC	208	NES
Tintinnopsis campanula	63	QR	32	NES
Tintinnopsis compressa	42	QR	324	NES
Tintinnopsis cylindrica	46	QR	46	NES
Tintinnopsis nana	196	QC	254	NES
Tintinnopsis parvula	71	QR	72	NES
Tintinnopsis radix	88	QC	48	NES
Tintinnopsis spp.	115	QC	900	NES
Undella subcaudata var. *acuta*	8	RR	32	OSS
Choreotrichida unid.	180	QC	670	

Table 3. Occurrence of each tintinnid species recorded in the studied period (1998-2016) in the Gulf of Trieste. Black squares indicate that the species was present at least once in the corresponding year. White squares indicate the absence of the species in those years.



ways < 12 from January to April, while it was > 25 from August to November (Figure 6). The Shannon Index (H') exhibited the same trend with values between 0.18 and 1.24 (Figure 6).

The pair-wise test showed a significant difference (p-perm < 0.05) in the community composition during the year. A significant pattern in composition similarity was detected and the highest similarity values were detected between contiguous months (Suppl. material 3).

The most representative species showed different abundance trends. *Stenosemella nivalis* reached the maximum average abundance (109 ± 537 ind. L^{-1}) in February while *S. ventricosa* in November (22 ± 65 ind. L^{-1}); both species presented the lowest values in August (< 2 ind. L^{-1}). The most common *Tintinnopsis* species showed the maxima in

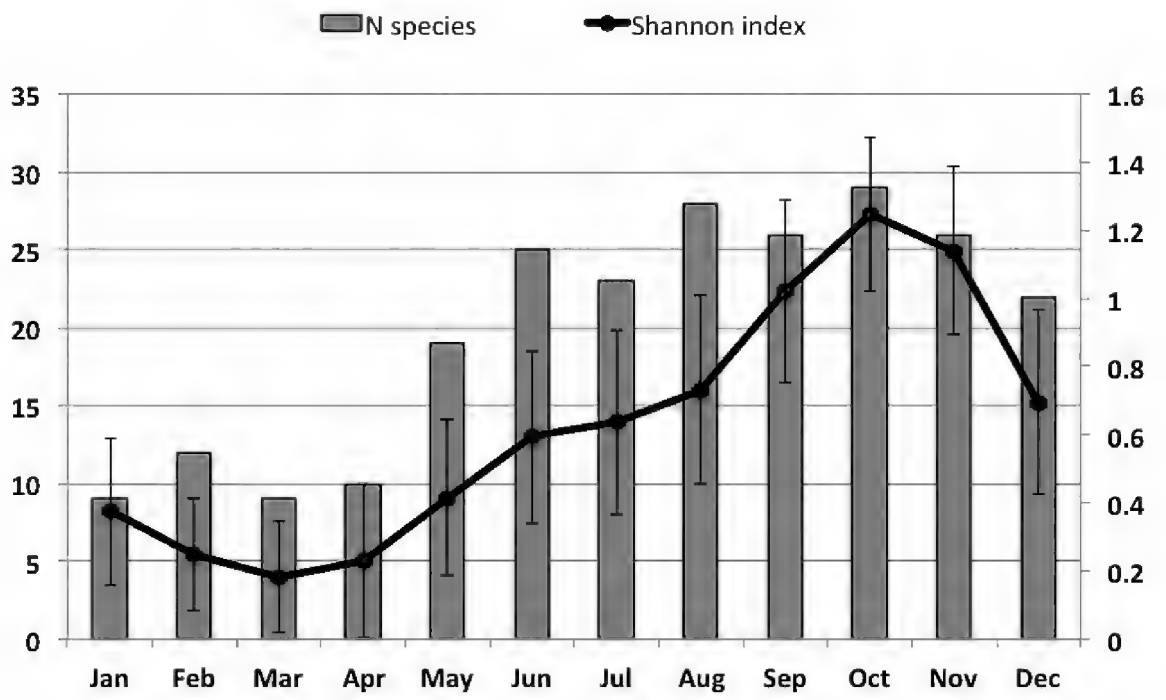


Figure 6. Seasonal patterns of tintinnid species richness (N species, left axis) and diversity index (Shannon Index + stdev, right axis) in the period from July 1998 to July 2016.

autumn. In particular, the data of the abundances integrated in the water column for *T. nana*, reached the average maximum value of 23 ± 43 ind. L^{-1} in October, followed by 11 ± 25 ind. L^{-1} in November. *Codonellopsis schabi* was present only from September to January reaching the maximum in November (16 ± 51 ind. L^{-1}) (Suppl. material 4). The hyaline genera presented the maximum average abundance from June to October. In this period, the two most common *Salpingella* species (*S. decurtata* and *S. rotundata*) showed a different trend. *Salpingella rotundata* reached the maximum average abundance in July (27 ± 143 ind. L^{-1}) while *S. decurtata* in October (8 ± 19 ind. L^{-1}). The genus *Eutintinnus* presented maxima in June or July. In particular, the most frequent species (*E. apertus*, *E. fraknoi* and *E. tubulosus*) showed maximum average abundance with values around 10 ind. L^{-1} in spring and summer (Suppl. material 4).

The results of IndVal test are synthesized in Table 4 where only the species which correlated significantly ($p < 0.05$) with each season are reported. These results confirm the seasonal pattern registered for the abundance. In particular, *S. nivalis* is the only species which significantly correlated to winter. All the species presenting high and significant IndVal values in summer are hyaline species. The majority of the species with high IndVal values in autumn are agglutinated species.

The tintinnid seasonal trend was strictly related to temperature and salinity values (PERMANOVA: pseudo-f = 1.1158 $p < 0.05$ for temperature and pseudo-f = 1.1531 $p < 0.01$ for salinity). The dbRDA plot highlighted the distribution of the samples on the base of the similarity in tintinnid composition and the two abiotic variables taken into consideration (Figure 7). The samples collected during winter and those collected during summer were antipodal distributed on the dbRDA plot, while the samples collected during spring and autumn showed a higher variability with respect to both salinity and temperature (Figure 7A).The total variation explained by dbRDA axes showed values of 6.8% for axis 1 and 0.3% for axis 2; the significance of axes

Table 4. List of tintinnid taxa and significant IndVal for each season; taxa are ordered by season and decreasing IndVal values. aut=autumn; spr=spring; sum=summer; win=winter.

	Season	IndVal	*p*-value
Codonellopsis schabi	aut	0.4307	0.001
Tintinnopsis nana	aut	0.3280	0.001
Stenosemella ventricosa	aut	0.2200	0.001
Tintinnopsis spp.	aut	0.2008	0.001
Tintinnopsis radix	aut	0.1933	0.001
Tintinnopsis beroidea	aut	0.1572	0.001
Dictyocysta elegans	aut	0.1410	0.001
Codonellopsis sp.	aut	0.1092	0.001
Tintinnopsis campanula	aut	0.1024	0.001
Salpingella decurtata	aut	0.0839	0.01
Tintinnopsis parvula	aut	0.0796	0.001
Amphorides quadrilineata	aut	0.0410	0.002
Amphorides laackmanni	aut	0.0319	0.001
Leprotintinnus nordqvisti	aut	0.0262	0.002
Eutintinnus tubulosus	spr	0.0912	0.008
Helicostomella subulata	spr	0.0642	0.024
Tintinnopsis compressa	spr	0.0623	0.002
Tintinnopsis cylindrica	spr	0.0413	0.003
Acanthostomella conicoides	spr	0.0361	0.022
Codonella sp.	spr	0.0273	0.012
Tintinnopsis angulata	spr	0.0150	0.029
Salpingella rotundata	sum	0.3105	0.001
Eutintinnus apertus	sum	0.1823	0.001
Metacylis sp.	sum	0.1076	0.001
Metacylis joergenseni	sum	0.1065	0.001
Eutintinnus fraknoi	sum	0.1020	0.001
Eutintinnus lusus-undae	sum	0.1006	0.001
Amphorellopsis acuta	sum	0.1002	0.001
Salpingella sp.	sum	0.0860	0.001
Undella subcaudata var. *acuta*	sum	0.0162	0.04
Favella sp.	sum	0.0132	0.044
Stenosemella nivalis	win	0.2882	0.001

and considered variables were tested via ANOVA.CCA ($p < 0.01$). The influence of temperature and salinity was also tested for each species. In Table S5 (Suppl. material 5), the Spearman's correlation values are presented. *S. nivalis* occurred with high abundance in the winter samples highlighting a clear negative correlation with temperature (Spearman value = -0.439) (Figure 7B). A strong positive correlation with temperature (0.34) was found for *S. rotundata* (Figure 7C) that reached the highest abundance in the summer samples. *Eutintinnus tubulosus* showed a weak but significant correlation with temperature (0.17) (Figure 7D) while *C. schabi* did not have any correlation with abiotic variables (Figure 7E). The Spearman's correlation values highlighted that salinity was negatively correlated with the majority of species and positively correlated only with *S. nivalis*.

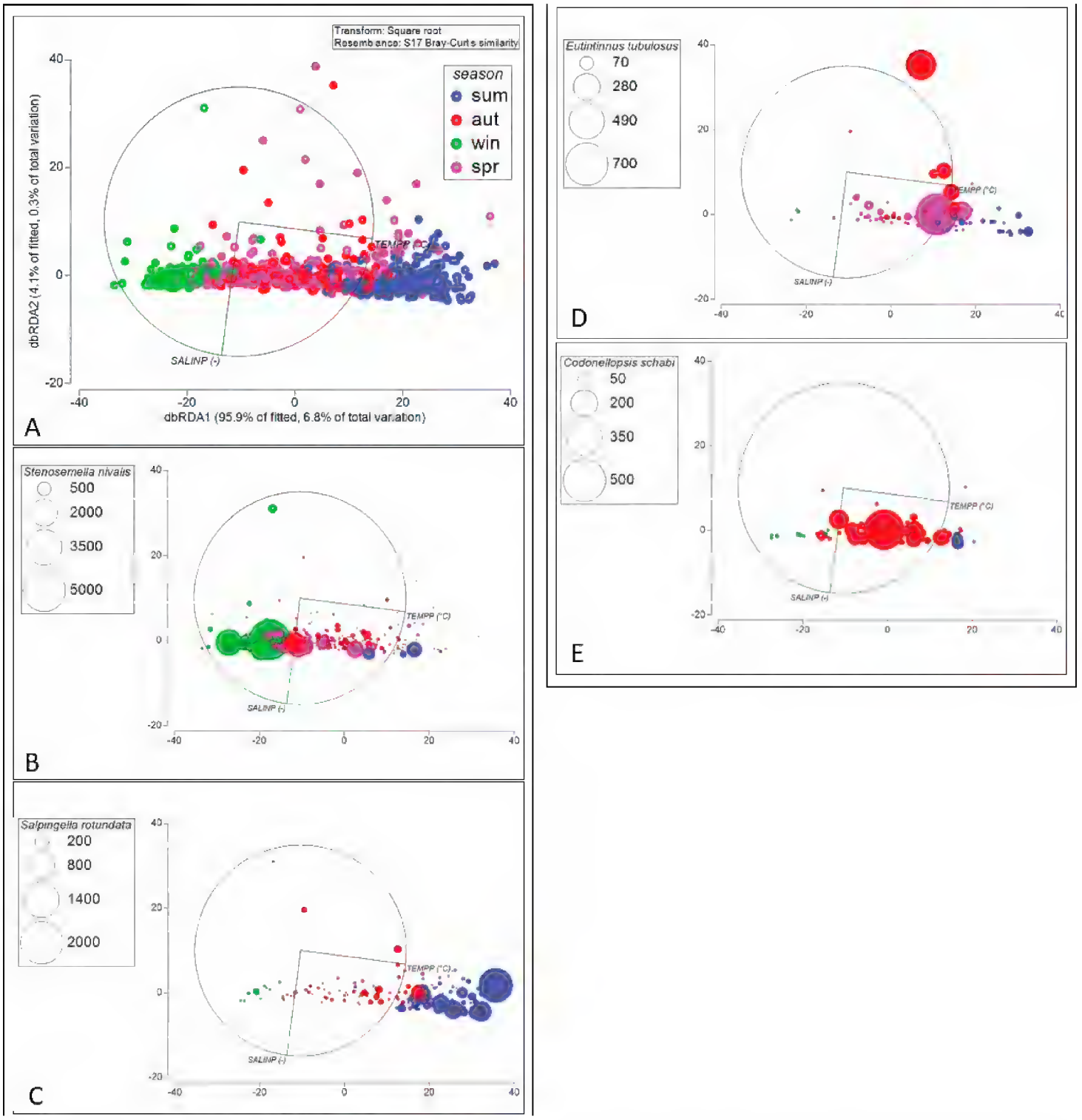


Figure 7. dbRDA (distance bases Redundancy Analysis) plot on the base of similarity in tintinnid composition constrained by temperature and salinity with axis significances (**A**). The colours represent samples collected in different seasons (blue=summer, red=autumn, green=winter, violet=spring). In the bubble plots (**B–E**), the bubble scale reports the relationship between bubble diameters and abundance measured for the most representative species: **B** *Stenosemella nivalis* **C** *Salpingella rotundata* **D** *Eutintinnus tubulosus* **E** *Codonellopsis schabi*.

Discussion

This study revealed that, in the Gulf of Trieste, tintinnid abundance integrated within the water column decreased remarkably after the beginning of the year 2000, followed by a continuous but slow recovery starting from the year 2007. This pattern matched that of phytoplankton biomass observed in the same area during the period 2001–2007, characterised by the reduction of river runoff, increase of surface salinity and decreasing concentration of nitrate and silicate (Giani et al. 2012, Mozetič et al.

2010, 2012). The reduction of river supply was due to a long period of drought, which can be foreseen as frequent in the near future due to the ongoing climatic changes.

The total tintinnid abundances were similar to those recorded in the same area by previous studies but higher to the rest of the Adriatic Sea. In the period 1986–1990, at the same station, but only for the surface, the tintinnid abundance reached a maximum value of about 1000 ind. L^{-1} (Monti et al. 2012). Fonda Umani et al. (2005a) registered values always below 200 ind. L^{-1} at 9 stations in the northern Adriatic Sea. In the middle Adriatic Sea, Bojanić et al. (2012) found values of about 100 cells L^{-1} at a station in the Split Channel, and Vidjak et al. (2009) reported a maximum of 3.3 ind. L^{-1} at a station along the Krka River estuary. In the open waters of the south Adriatic Sea, Kršinić and Grbec (2006) found values with a maximum of 33 ind. L^{-1}. The higher abundance values found in the Gulf of Trieste, compared to the middle and south Adriatic, reflect the patterns of the other plankton community compartments in the area, which are characterised by high standing stock but low diversity (Fonda Umani 1996).

The total tintinnid abundance, integrated within the water column, showed relevant inter-annual fluctuations characterised by autumn maxima and, only in few cases, summer peaks. All the maxima, both in summer and in autumn, were due to blooms of *S. nivalis* and other few species. The isolated abundance peaks of some neritic species, such as *S. nivalis*, might be explained by the seasonal recruitment from cysts, as consequence of particular environmental conditions (Kamiyama 2013). The dense lorica of *Stenosemella* genus, agglutinated with mineral particles, may in fact allow the species belonging to this genus to rapidly sink to the bottom, where they can lie as cysts (Capriulo et al. 1982). The excystment of these resting stages can play an important role in recruiting new tintinnid populations in the water column (Paranjape 1987). Very unfortunately, this hypothesis could not be tested in our study because cysts were not considered in the microscopic analyses. In June 2015 (15 m) and July 2015 (5 m), the high abundances were due to *Helicostomella subulata* and *S. rotundata*, respectively. The abundance peaks of some oceanic species, such as *S. rotundata*, can be explained by the advection of water masses from the middle and south to the northern Adriatic (Kršinić 2010b). In fact, *S. rotundata* is primarily distributed in the surface layer of the Otranto Strait and in the open waters of the south Adriatic (Kršinić 2010b). Conversely, *H. subulata* is considered an estuarine species very rare or absent in the open waters of south and middle Adriatic (Kršinić 2010b). This species was quite rare in our samples and only occasionally bloomed in spring. *Helicostomella subulata* was reported as abundant during the warmer months in the south-east part of the Gulf of Trieste by Lipej (1992), and along the Adriatic coast in relation to river inputs (Kršinić 1987, Bojanić et al. 2012) or in proximity to harbors (Moscatello et al. 2004).

The seasonal pattern registered in this study differed between agglutinated and hyaline species. Tintinnids build the lorica according to the material available in the water column and the two lorica types, in general, correspond to different habitats: agglutinated lorica to coastal environments and hyaline lorica to open waters. In our time series, the open sea species were more numerous (27) than the neritic ones (20). The former were more abundant in summer, in agreement with the worldwide pattern

of hyaline loricae dominating the communities in summer and agglutinated loricae in winter (Dolan and Pierce 2013). Species with agglutinated loricae may be better adapted to turbulent conditions, which are more frequent in the winter season (Dolan and Pierce 2013). In the Adriatic Sea, agglutinated species dominate along the coastal areas while hyaline species characterise the open sea areas of middle and southern Adriatic (Kršinić and Precali 1997). The presence of oceanic species in the Northern Adriatic Sea can therefore be connected to the advection of higher salinity waters from the middle Adriatic.

At station C1, we found the four most widely distributed and reported species in the world: *A. quadrilineata*, *D. ganymedes*, *S. steenstrupii* and *E. apertus* (Dolan and Pierce 2013). However, with the exception of *E. apertus*, they were rare or very rare in the Gulf of Trieste. On the contrary, *S. nivalis*, *S. ventricosa* and *T. beroidea* were recorded in all the years considered. These species are common in the whole Mediterranean Sea (Dolan 2000) and they are often dominant in coastal tintinnid communities (Fonda Umani et al. 2010). In the Gulf of Trieste, they can be considered as keystone species and their presence and fluctuation in abundance must be considered an important signal of possible changes in the whole plankton community. In a study conducted from June 1999 to July 2002, *S. nivalis* and *T. nana* were present at 9 stations in 3 transects across the northern and central Adriatic Sea (Fonda Umani et al. 2005a). The southern transect was characterised by *D. ganymedes*, *E. lusus-undae*, *X. longicaudata* and *R. spiralis*, typical of warmer and salty waters. In our study, *R. spiralis* and *D. ganymedes* were recorded only in few years and can be considered rare species. Their presence in the northern part of the Gulf of Trieste can suggest a possible previous water intrusion from southern areas.

In our study, tintinnids were represented by 19 genera and 35 species. This number of taxa agreed with other studies within the same area. Lipej (1992) found 30 species in the southeast part of the Gulf of Trieste, along the Slovenian coast, from 1989 to 1991. Kršinić (2010b) found 50 species for all the North Adriatic Sea, with a large number of oceanic species (30). In the period 1998–2010, Monti et al. (2012) recorded 40 species in the surface samples of the same site. The lower number of species in the present study in comparison to Monti et al. (2012) can be justified by the revision of taxonomy that we applied more recently to our dataset following Kršinić (2010a). As indicated in Methods, we also shifted to the genus level some species that were very rare and/or with identification problems, which caused a reduction of species number. In comparison to other time series of tintinnids in coastal areas of the Mediterranean Sea, the number of species we found in this study was generally lower. For example, in the Northern Ionian Sea, Sitran et al. (2007) examined the tintinnid community at a fixed station for two years (2003–2004) and found 79 species belonging to 23 genera. At a station in the Southern Tyrrhenian Sea, the same authors (Sitran et al. 2009) found 67 species belonging to 22 genera. At a coastal site in the Gulf of Naples, 57 species were identified in 4 years (Modigh and Castaldo 2002), but only seven accounted for 81% of total numbers (*T. minuta*, *T. beroidea*, *Metacylis annulifera*, *E. tubulosus*, *H. subulata*, *S. curta* and *S. decurtata*). The lower number of tintinnids species found in this study, in comparison to other areas of the Mediterranean Sea, reflects the reduction in the

number of species for all planktonic compartments observed in the Gulf of Trieste due to the extreme variability of the environmental conditions of the area (Ingrosso et al. 2016). The hydrodynamic features of the Gulf of Trieste are mostly controlled by pulses of external inputs (e.g., terrigenous material, fresh waters, middle Adriatic water advection), that may alter the phytoplankton successions (Cataletto et al. 1995, Fonda Umani 1996). In particular, the high and unpredictable variability of the river inputs cause remarkable variability in salinity values (from 33 to 38) (Malačič et al. 2006) and nutrient concentrations (e.g., from 2 to 84.2 μmol L^{-1} for N/NO_3, and from 0.05 to > 3 μmol L^{-1} for P/PO_4) (Cataletto et al. 1995). Ample is also the range of sea surface temperature that shows a seasonal cycle from winter minima of 6°C to summer maxima of >29°C (Celio et al. 2006).

Generally, the lowest species richness and diversity in the Mediterranean tintinnid assemblages is recorded in summer months, reflecting the seasonal minimum of primary production (Dolan and Pierce 2013). In contrast, in our study, the lowest species richness occurred in winter (8–12 species) probably due to the extreme cold temperature and high salinity in this season (Ingrosso et al. 2016). The highest species richness values were detected in the autumn, in accordance with other studies in costal and confined areas. Bojanić et al. (2012) detected higher numbers of tintinnid species and high diversity (H' = 0.42–2.71) at a costal Adriatic station, between September and November. In the southern Adriatic Sea (from January 1980 to May 1990), Kršinić and Grbec (2006) detected the highest number in October. For the Ionian Sea the highest value of diversity index (H' = 1.3–2.7) for tintinnids was found in September and November (Sitran et al. 2007).

In our study, the differences in the vertical distribution of tintinnid composition were not significant, as reported for similar depths by Sitran et al. (2007). However, we observed significant differences in the relative abundance of the most common species among the different depths. The water column in the Gulf of Trieste, as already stated, is subject to strong variations in temperature, salinity, and vertical stratification during the year (Malačič and Petelin 2001). During spring, the freshwater input and surface heating cause the thermohaline stratification, which increases in strength during summer. In autumn and winter, convective and mechanical mixing, induced by water cooling and wind, disrupts the vertical stratification leading to a mostly homogeneous water column. Contrary to our expectation, the increase of vertical stability in summer did not enhance the vertical differences in abundance of the key species. This result may suggest that tintinnids abundances are more linked to the food availability, i.e. phytoplankton at the surface and re-suspended organic matter at the bottom.

The seasonal pattern of tintinnid species can be strictly related to temperature and salinity. Tintinnid diversity appeared to be positively linked to salinity at a coastal station in the Ionian Sea (Sitran et al. 2007) and along the Krka river estuary (Vidjak et al. 2009), whilst Dolan and Gallegos (2001) highlighted how tintinnid diversity in the estuarine environment was high and increased with decreasing salinity. The difference among the results of these authors may depend on the environmental factors that characterize the different studied areas. Temperature dependent distribution of different tintinnid species has been reported in the middle Adriatic (Bojanić et al. 2012) as

well as in other areas (Abboud-Abi Saab 1989, Koray and Őzel 1983, Posta 1963). In our work, we found that temperature showed direct or inverse correlation according to species, while salinity was negatively correlated with the majority of species. These results suggest that temperature may be more relevant than salinity in shaping the tintinnid community. The abundance of most tintinnids was related to temperature, but the particular response of each tintinnid to the environmental factors was species specific, as found by Kamiyama and Tsujino (1996) in Hiroshima Bay. In our work, the variation in temperature correlation was strictly related to the species seasonality and consequently to their presence and abundance. Therefore, the expected global increase in temperature could have, in the future, an important effect on the tintinnid community in the Gulf of Trieste.

Conclusions

This paper presented the characteristics of tintinnid assemblages along an 18-year period at a coastal site of the Gulf of Trieste that belongs to the Italian LTER (Long Term Ecological Research) network. Our long-term study has highlighted clear seasonal patterns and large interannual fluctuations of diversity and abundance. Three species appeared to be as keystone species for their persistent occurrence and relevant abundance and can be used to monitor the long-term evolution of the whole microzooplankton community in the Gulf of Trieste. The lack of significant differences among the community composition and relative abundance between 5 and 10 meter depths may suggest to reduce the sampling effort to the surface and bottom depths to monitor the tintinnid assemblages in this shallow marine area.

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Supplementary material 1

Table S1. Result of pair-wise test on factor year

Authors: Marina Monti-Birkenmeier, Tommaso Diociaiuti, Serena Fonda Umani

Data type: statistical data

Explanation note: The years: 1999–2000; 1999–2001; 2003–2005; 2005–2006; 2008–2010; 2012–2014, did not show significant difference

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Link: https://doi.org/10.3897/natureconservation.34.29841.suppl1

Supplementary material 2

Table S2. Occurrence for each tintinnid species recorded in the studied period (1998–2016) in the Gulf of Trieste at each sampling depth (A, surface; B, 5 m; C, 10 m; D, 15 m)

Authors: Marina Monti-Birkenmeier, Tommaso Diociaiuti, Serena Fonda Umani

Data type: occurence

Explanation note: Black squares indicate that the species was present at least once in the corresponding year. White squares indicate the absence of the species in those years.

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Supplementary material 3

Table S3. Result of pair-wise test on factor month

Authors: Marina Monti-Birkenmeier, Tommaso Diociaiuti, Serena Fonda Umani

Data type: statistical data

Explanation note: The months: Dec-Jan; Dec-Feb; Jan-Feb; Jan-Mar; Feb-Mar, did not show significant differences.

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Supplementary material 4

Figure S4. Abundance trend of the most representative agglutinated species: *S. nivalis, S. ventricosa, T. nana, C. schabi* and hyaline species: *S. rotundata, S. decurtata, E. fraknoi, E. apertus*

Authors: Marina Monti-Birkenmeier, Tommaso Diociaiuti, Serena Fonda Umani

Data type: abundance

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Link: https://doi.org/10.3897/natureconservation.34.29841.suppl4

Supplementary material 5

Table S5. Sperman's correlation values with temperature and salinity for each tintinnid taxon detected in the studied period

Authors: Marina Monti-Birkenmeier, Tommaso Diociaiuti, Serena Fonda Umani

Data type: statistical data

Explanation note: in red the significative correlation $p < 0.05$

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